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Crystallography of α-Lactalbumin

III.† Crystals of Baboon Milk α-Lactalbumin

Crystals of baboon α-lactalbumin suitable for structure analysis at high resolution have been obtained. They have cell dimensions $a = 35{\cdot}5$ Å, $b = 69{\cdot}1$ Å, $c = 46{\cdot}1$ Å, space group $P2_12_12$ and contain one molecule of α-lactalbumin per asymmetric unit.

During the last decade the milk protein α-lactalbumin has received much attention from research workers interested, in particular, in two novel aspects: its participation in the synthesis of lactose, by combining with a galactosyl-transferase to form lactose synthetase, a unique enzyme system, reviewed in detail by Brew & Hill (1975); and the similarity of its primary structure to that of lysozyme, a similarity that may well extend to the three-dimensional structure (Browne *et al.*, 1969; Warme *et al.*, 1974). With the aim of examining this relationship in detail, and as an essential step towards understanding the activity of lactose synthetase, attempts at direct determination of the tertiary structure of α-lactalbumin by X-ray crystallography were initiated a number of years ago (Aschaffenburg *et al.*, 1972*a*,*b*). Suitable crystals were not obtained from cow's milk, and promising crystals of goat α-lactalbumin proved difficult to analyse. α-Lactalbumins from several other species were investigated (Fenna, 1973), including human α-lactalbumin which, except in one non-reproducible instance, failed to crystallize in our hands. It was somewhat surprising, therefore, to find that the protein isolated from the milk of another primate, the baboon (*Papio cynocephalus*) crystallised with ease, and eventually in a form suitable for high-resolution work. Subsequent work has shown that the amino acid sequence of baboon α-lactalbumin is quite similar to the sequence of human α-lactalbumin determined by Findlay & Brew (1972). Only one difference is observed in the N-terminal 30 residues, and two of the three peptides produced by cyanogen bromide cleavage have compositions identical to those of equivalent regions of the human protein (R. Greenberg & M. L. Groves, E.R.R.C., Philadelphia, personal communication).

Primate milks contain both α-lactalbumin and lysozyme in relatively high concentration (Buss, 1971) and thus make possible the isolation of the two proteins from the same source for potentially more clear-cut comparisons than might be expected from comparisons of the two proteins from widely separated species. (Cow α-lactalbumin and hen egg-white lysozyme, being easily accessible, are the most commonly used representatives of their respective classes in most research on these proteins.) While located at the Southwest Foundation for Research and Education, San Antonio, Texas, one of us (D. H. B.) devised a method for the successive isolation of the two proteins from baboon milk. The lysozyme was prepared and characterised (Buss, 1971), and has been used subsequently in the determination of the primary structure (Hermann *et al.*, 1973) and in an analysis of its crystal structure at 6 Å resolution (Fenna, 1973).

† Paper II in this series is Aschaffenburg *et al.* (1972*b*).

Freeze-dried "delysozymed" samples served as the starting material for the isolation of two batches of α-lactalbumin of 40 and 90 mg, respectively, which were used in the initial crystallographic studies. The protein was obtained by application of the appropriate steps of the general method of Quarfoth & Jenness (1975), which consists essentially of column treatment of whey on Sephadex G100 and DEAE/Sephadex A50. Later a larger sample of over 0·5 g of α-lactalbumin was prepared from 300 ml of fresh baboon milk obtained from the San Antonio Centre. This isolation procedure differed in that only one column treatment, on Sephadex G100, was applied.

(a) *Crystallization*

Crystals of the α-lactalbumin were prepared by a salting-out procedure with ammonium sulphate similar to that used for goat α-lactalbumin (Aschaffenburg *et al.*, 1972*b*). In the most successful experiments, 3% (w/v) solutions in 0·2 M-phosphate buffer (pH 6·8) were mixed with saturated ammonium sulphate solution (adjusted to the same pH) so as to cover a range of saturation from 45% to 50%. (These conditions differ from those used initially and recorded by Fenna, 1973.) As with the goat α-lactalbumin, flocculant precipitates form, whose presence appears to be essential for subsequent crystal formation. Crystals appear most quickly at the high salt concentration but grow slowly, taking four to six weeks to attain full size. They can then be transferred to a protein-free solution made up of 50% saturated ammonium sulphate in phosphate buffer. The nature of the buffer seems to be of critical importance in the preparation of good crystals: replacement of the phosphate by Tris·HCl or imidazole buffer resulted in the formation of amorphous precipitates or inferior crystals.

The first crystals, prepared from the delysozymed fraction of baboon milk, consisted mainly of small multiples together with a few single crystals suitable for X-ray analysis, none of which was large enough for diffractometry. Crystals grown from the α-lactalbumin sample prepared from fresh milk failed likewise to attain the size needed for diffractometry if they were grown directly from solutions of the freeze-dried protein. If the protein was first salted out with ammonium sulphate, however, and then set up to crystallize after dialysis of the precipitate against the phosphate buffer, diamond-shaped plates of varying thickness were formed with maximum dimensions of 2·0 mm × 0·8 mm × 0·4 mm. The formation of such large crystals was first observed in recovery experiments. It did not occur with the original delysozymed material.

(b) *Crystal data*

Crystals of baboon α-lactalbumin are diamond-shaped plates in the orthorhombic spheroidal class with the pinacoids {010} best developed and bounded by the domes {101}. Precession photographs show a strong diffraction pattern extending beyond 2·0 Å spacings and systematic absences that are characteristic of the space group $P2_12_12$. The unit cell dimensions of the first small crystals derived from the delysozymed milk were $a = 33{\cdot}6 \pm 0{\cdot}1$ Å, $b = 69{\cdot}6 \pm 0{\cdot}1$ Å, $c = 47{\cdot}0 \pm 0{\cdot}1$ Å, giving a unit cell volume $V = 1{\cdot}099 \times 10^5$ Å^3 (Fenna, 1973). The larger crystals, produced from the later supply of α-lactalbumin, have cell dimensions $a = 35{\cdot}5 \pm 0{\cdot}1$ Å, $b = 69{\cdot}1 \pm 0{\cdot}1$ Å, $c = 46{\cdot}1 \pm 0{\cdot}1$ Å, giving $V = 1{\cdot}13 \times 10^5$ Å^3. This difference is not yet understood. Subsequent work has been done with the latter crystals.

The density of these crystals, measured on a bromobenzene/xylene density gradient

calibrated with drops of caesium chloride solution of known concentration, was 1·23 g/cm^3. Given that the molecular weight of α-lactalbumin is approximately 14,400, these data show that there are four molecules per unit cell, with $V_m = 1{\cdot}96$ Å^3 per dalton (Matthews, 1968), and that the mother liquor comprises 33% (w/w) of the crystal.

These are the first crystals of α-lactalbumin to be reported that contain one molecule per asymmetric unit (Fenna, 1973), and the analysis of their structure is now well advanced.

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